(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 136 558 A1

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
26.09.2001 Bulletin 2001/39

(51) Int Cl.[7]: **C12N 15/31**, C12N 15/82, C07K 14/195, A01H 5/00

(21) Application number: **00870051.0**

(22) Date of filing: **22.03.2000**

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

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Remarks:
The sequence listing, which is published as annex to the application documents, was filed after the date of filing. The applicant has declared that it does not include matter which goes beyond the content of the application as filed.

(54) Genetically modified plants and plant cells comprising heterologous heavy metal transport and complexation proteins

(57) The present invention relates to genetically modified plants and plant cells, comprising nucleotide sequences encoding heterologous heavy metal transport protein.

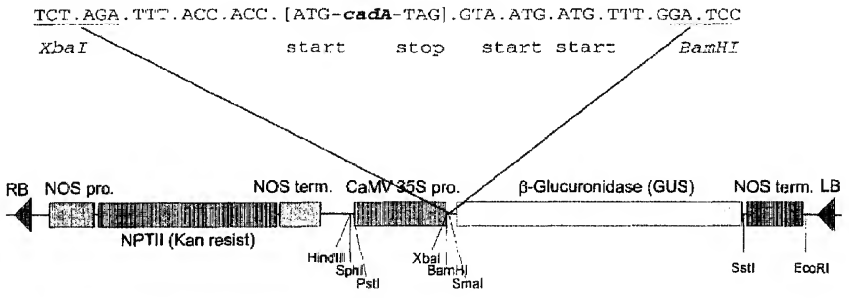

Fig. 1

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Description

Field of the invention

[0001] The present invention is in the field of genetically modified plants and plants cells having improved heavy metal tolerance and accumulation due to increased plant growth and biomass production based upon the expression of exo-cytoplasmic heavy metal resistance system (efflux and complexation).

[0002] More particularly, the present invention is related to genetically modified plants and plant cells, comprising nucleotide sequences encoding heterologous heavy metal transport proteins and exocytoplasmic metal binding proteins of various origins.

Background of the invention and state of the art

[0003] Heterologous nucleic acid sequences, coding for heavy metal resistance, were functionally expressed in plants, to improve their tolerance against these toxic elements. The heterologous heavy metal resistance genes, in casu represent either heavy metal efflux systems or functions involved in heavy metal sequestration.

[0004] Until present, only cytoplasmic functions that provide increased heavy metal resistance were expressed in plants :

1. Expression of heterologous metallothionein and phytochelatines in plants

[0005] Metallothioneins and phytochelatines, which are rich in cystein sulfhydryl residues that bind and sequester heavy metal ions in very stable complexes (Karin, 1985), are found in eukaryotic organisms, but recently also in *Synechococcus.* Various MT genes - mouse MTI, human MTIA (alpha domain), human MTII, Chinese hamster MTII, yeast CUP1, pea PsMTA - have been transferred to tobacco, cauliflower or *Arabidopsis thaliana* (Lefebre et al., 1987; Maiti et al., 1988, 1989, 1991; Misra and Gedamu, 1989; Evans et al., 1992; Yeargan et al., 1992; Brandle et al., 1993; Pan et al., 1993; Elmayan and Tepfer, 1994; Hattori et al., 1994; Pan et al., 1994a, b; Hasegawa et al., 1997). As a result, varying degrees of enhanced Cd tolerance have been achieved, being maximally 20-fold compared with the control. Metal uptake levels were not dramatically changed; in some cases there were no differences, in others maximally 70% less or 60% more Cd was taken up by the shoots or leaves. Only one study has been reported on a transgenic plant generated with MT of plant origin. When pea (*Pisum sativum)* MT-like gene PsMTA was expressed in *Arabidopsis thaliana*, more Cu (several-fold in some plants) accumulated in transformed than in control plants (Evans et al., 1992).

2. Heterologous expression of heavy metal reduction

[0006] The only example known is the *mer* operon of *Tn21* of *Shigella* flexneri, whose expression in plants results in the reduction mercury (Hg^{2+}) in its metallic form (Hg^{0}). This metallic mercury is volatilized out of the cell (Rugh *et al.* 1996).

Aims of the invention

[0007] The present invention aims to provide a new way in obtaining plants and plant cells with improved heavy metal tolerance characteristics, and possibly heavy metal accumulation.

[0008] Another aim of the present invention is to provide such plants and plant cells which allow increased heavy metal resistance for revegetation and phytostabilisation of heavy metal contaminated sites.

[0009] A further aim of the present invention is to provide plants and plant cells, characterised by increased heavy metal accumulation combined with increased heavy metal tolerance which allow phytoextraction of heavy metals (inclusive rhizofiltration).

[0010] A last aim of the present invention is to provide a method which results in the possibility to improve important agriculture crop species with high biomass production in their heavy metal tolerance and accumulation.

Summary of the invention

[0011] The present invention is related to genetically modified plants and plant cells, comprising nucleotide sequences encoding one or more heterologous heavy metal transport and/or sequestration proteins of various prokaryotic or eukaryotic origins.

[0012] Said transporters are preferably membrane proteins, which result in reduced toxicity due to the efflux of heavy metals from the cells, being preferably selected from the group consisting of P-type ATPases, 3 component efflux

pumps, ABC transporters and CDF proteins (Cation Diffusion Facilitator proteins).

[0013] The family of the P-type ATPases is preferred, because of their advantage that for functional resistance only one protein is required.

[0014] Said proteins are found in both prokaryotic and eukaryotic organisms including plants.

[0015] Another advantage of said transporters is found as resistance mechanisms against many toxic trace elements of environmental concern, such as copper, cadmium, lead, zinc and silver.

[0016] Unexpectedly, it was not necessary to make structural changes in the coding sequence of said proteins, like it is necessary for the merA gene in order to obtain functional expression in plants (Rugh et al., 1996).

[0017] Preferably, the gene incorporated in the plants or plant cells is a gene encoding a bacterial P-type ATPase, preferably the cadmium ATPase, such as the *cadA* gene.

[0018] According to a second embodiment of the present invention, the system is based upon a prokaryotic heavy metal sequestration system, such as the *pcoA* family protein (more preferably the *pcoA* gene).

[0019] The various nucleotide sequences encoding heterologous heavy metal transport proteins can be deleted partially from non-specific nucleotide sequences which are not involved in efficient heavy metal transport or accumulation.

[0020] Said genetic sequences could be incorporated in a vector for the transfection of said plants or plant cells, such as the pBI121 vector, as described in the figure 1, said vector being advantageously an *E. coli/Agrobacterium/* plant shuttle vector, said vector comprising preferably a CaMV 35S promoter (a strong promoter constitutively expressed in plants).

[0021] Preferably, the system was introduced in the plants, such system allowing the transformation of plants with the *Agrobacterium tumefaciens* technology.

Short description of the drawings

[0022] Fig. 1 is a schematic representation of the cloning of *cadA* in pBI121.

[0023] Fig. 2 is a leaf disk-test with Nt WT SR1 (wild type), Nt PBI14 (pBI121) and Nt Cd 309 (pBI121-*cadA*) on 350 µM Cd and control medium without Cd.

[0024] Fig. 3 represents the regeneration and growth of Nt WT SR1 (wild type), Nt PBI14 (pBI121) and Nt Cu122 (pBI121-pcoA) on 100 µM Cu, the plant growth being shown from above (left) and top (right).

Detailed description of the invention

Heterologous expression of *cadA*

[0025] The heavy metal efflux system was CadA, a member of the P-type heavy metal efflux ATPase family of proteins found both in prokaryotic and eukaryotic organisms. P-type ATPases are all cation pumps, either for uptake, for efflux or for cation exchange. These enzymes have a conserved aspartate residue that is transiently phosphorylated from ATP during the transport cycle, hence the name 'P-type' ATPase (Silver *et al.*, 1993).

[0026] The *cadA* gene from *Staphylococcus aureus* was amplified by PCR and cloned in the pBI121 vector.

[0027] During PCR, appropriate plant specific translation signals were added as well as XbaI and BamHI restriction sites, allowing cloning of the insert in the correct orientation.

[0028] The *cadA* fragment was cloned in the *Escherichia coli/Agrobacterium/plant* shuttle vector pBI121. In this vector, *cadA* expression is derived from the CaMV35S promotor, a strong promoter constitutively expressed in plants. The system was introduced in the plant *Nicotiana tabacum* cv. Petit Havana line SR1 via an *Agrobacterium tumefaciens* transformation (Horsch *et al.*, 1985). The selection marker used was kanamycine.

[0029] Kanamycine resistant transformants were obtained after transformation. All the kanamycine resistant transformants tested showed an increased resistance to cadmium (tested by a leaf disk assay) compared to the wild type and transformant with the pBI121 vector without gene (fig. 1). This proves that the CadA P-type ATPase can be functionally expressed in plants, resulting in an increased resistance of the plant to the trace element (in casu cadmium).

[0030] It can be expected that for other members of the P-type ATPase family, which form a family of closely related proteins (both structural and functional) the same positive effect on resistance to specific trace elements will be found. Until present, P-type ATPases from both prokaryotic and eukaryotic have been identified that were found to interact with Zn, Cd, Pb, Cu and Ag (see table 1). It can not be excluded that P-type ATPases, encoding resistance to other trace elements including radioisotopes, will be identified.

Table 1:

different representatives of the family of P-type ATPases, from prokaryotic and eukariotic origin, which encode resistance against trace elements such as Zn, Cd, Pb, Cu and Ag.			
Gene	**Sequence ID**	**Metals**	**Reference**
CadA	P20021	Cd, Zn and Pb	Nucifora *et al.* 1989 Rensing *et al.* 1998
ZntA	P37617	Zn and Pb	Rensing *et al.* 1997 Rensing *et al.* 1998
CopF	Non available	Cu	van der Lelie and Borremans unpublished
PbrA	Not available	Pb	Borremans et al, 2000
SilP	AF067954, nucleotide sequence *sil* operon	Ag	Gupta *et al,* 1999
Menkes' disease	Q04656	Cu	Vulpe *et al.* 1993
Wilsons' disease	U08344	Cu	Pethrukin et al. 1993

Heterologous expression of *pcoA*

[0031] The other heavy metal resistance system is involved in exo-cytoplasmic heavy metal sequestration. The tested gene here was *pcoA* from *Escherichia coli* (Brown *et al.,* 1995), which was also cloned in pBI121 and introduced in *Nicotiana tabacum* through an *Agrobacterium tumefaciens* transformation in a way similar as described for *cadA*. Kanamycine resistant transformants were obtained after transformation. All the kanamycine resistant transformants tested showed an increased resistance to copper (tested by a leaf disk assay) compared to the wild type and transformant with the pBI121 vector without gene (Fig. 3).

[0032] The *pcoA* protein has many closely related members, found to be involved in resistance against Cu. In addition, other proteins of these copper resistance determinants have also been shown to be involved in Cu sequestration, such as PcoC/CopC and CopE. These proteins, although different in structure, are also active in the bacterial periplasm and possess similar heavy metal binding sites as *pcoA*. In addition, a CopE like protein, referred to as SilE, was identified in the *Salmonella sil* operon encoding for Ag-resistance. The potential genes whose heterologous expression can result in improved resistance, are summarised in table 2.

Genes	**Sequence ID**	**Metals**	**References**
cop operon (*copA*, C) e.g. of *Pseudomonas syringae*	M19930	Cu	Mellano and Cooksey (1988)
pco operon (*pcoA*, C) of e.g. *E. coli*	G619126	Cu	Brown *et al.,* 1995
PcoE	X83541	Cu	Brown *et al.,* 1995
sil operon of *Salmonella*	AF067954, nucleotide sequence *sil* operon	Ag	Gupta *et al.,* 1999

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Annex to the application documents - subsequently filed sequences listing

[0034]

SEQUENCE LISTING

<110> Vlaamse Instelling voor Technologisch Onderzoek (V

<120> GENETICALLY MODIFED PLANTS AND PLANT CELLS COMPRISING HETEROLOGOUS HEAVY METAL TRANSPORT AND COMPLEXATION PROTEINS

<130> P.VITO.25/EP

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Met Asn Val Tyr Arg Val Gln Gly Phe Thr Cys Ala Asn Cys Ala Gly
         15                  20                  25

aag ttt gag aaa aat gtt aaa aag att cca ggc gtt cag gac gca aaa   147
Lys Phe Glu Lys Asn Val Lys Lys Ile Pro Gly Val Gln Asp Ala Lys
     30                  35                  40

gta aac ttt ggc gct tct aaa att gat gta tat gga aat gca tcg gtt   195
Val Asn Phe Gly Ala Ser Lys Ile Asp Val Tyr Gly Asn Ala Ser Val
 45                  50                  55                  60

gaa gaa ctt gaa aaa gca ggt gct ttt gag aat cta aaa gta tct cct   243
Glu Glu Leu Glu Lys Ala Gly Ala Phe Glu Asn Leu Lys Val Ser Pro
                 65                  70                  75

gaa aaa cta gcg aat caa acg ata caa agg gtt aaa gat gac act aag   291
Glu Lys Leu Ala Asn Gln Thr Ile Gln Arg Val Lys Asp Asp Thr Lys
             80                  85                  90

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Ala His Lys Glu Glu Lys Thr Pro Phe Tyr Lys Lys His Ser Thr Leu
         95                  100                 105

ctg ttt gcc aca cta cta att gct ttt ggt tac ctt tct cac ttt gta   387
Leu Phe Ala Thr Leu Leu Ile Ala Phe Gly Tyr Leu Ser His Phe Val
    110                 115                 120

aat gga gaa gat aac ctc gta act tcc atg tta ttt gta ggt tct att   435
Asn Gly Glu Asp Asn Leu Val Thr Ser Met Leu Phe Val Gly Ser Ile
125                 130                 135                 140

gta att ggc gga tat tca tta ttt aaa gtc ggt ttt caa aat ttg ata   483
Val Ile Gly Gly Tyr Ser Leu Phe Lys Val Gly Phe Gln Asn Leu Ile
                145                 150                 155

cgc ttt gat ttc gac atg aaa acc ctg atg acc gtt gcc gtt att gga   531
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Phe Ala Ile Ser Glu Ala Leu Glu Arg Phe Ser Met Asp Arg Ser Arg
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205                 210                 215                 220

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                225                 230                 235

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Gly Asp Ile Met Ile Val Lys Pro Gly Glu Lys Ile Ala Met Asp Gly
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    270                 275                 280

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285                 290                 295                 300

gaa gat aca acc att acc aag att att cat ctt gtt gaa gaa gca caa   963
Glu Asp Thr Thr Ile Thr Lys Ile Ile His Leu Val Glu Glu Ala Gln
                305                 310                 315

ggg gag cgt gct cca gcc caa gca ttc gtt gat aaa ttt gcg aaa tac   1011
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            320                 325                 330

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    350                 355                 360
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365                 370                 375                 380

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Ile Ser Ile Val Ser Ala Ile Gly Asn Ala Ala Lys Lys Gly Val Leu
                385                 390                 395

gtt aaa ggt ggt gtc tat ctc gag aaa tta gga gcc att aag aca gtc   1251
Val Lys Gly Gly Val Tyr Leu Glu Lys Leu Gly Ala Ile Lys Thr Val
            400                 405                 410

gca ttt gat aaa aca gga aca ctg aca aaa ggt gta cca gtg gta aca   1299
Ala Phe Asp Lys Thr Gly Thr Leu Thr Lys Gly Val Pro Val Val Thr
        415                 420                 425

gat ttt gaa gta tta aat gac caa gtg gaa gaa aaa gag cta ttc tct   1347
Asp Phe Glu Val Leu Asn Asp Gln Val Glu Glu Lys Glu Leu Phe Ser
    430                 435                 440

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            480                 485                 490

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        495                 500                 505

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Thr Gly Asp Asn Gln Gly Thr Ala Asn Ala Ile Gly Thr His Val Gly
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         35                  40                  45

Ala Ser Lys Ile Asp Val Tyr Gly Asn Ala Ser Val Glu Glu Leu Glu
     50                  55                  60

Lys Ala Gly Ala Phe Glu Asn Leu Lys Val Ser Pro Glu Lys Leu Ala
 65                  70                  75                  80

Asn Gln Thr Ile Gln Arg Val Lys Asp Asp Thr Lys Ala His Lys Glu
                 85                  90                  95

Glu Lys Thr Pro Phe Tyr Lys Lys His Ser Thr Leu Leu Phe Ala Thr
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Leu Leu Ile Ala Phe Gly Tyr Leu Ser His Phe Val Asn Gly Glu Asp
        115                 120                 125

Asn Leu Val Thr Ser Met Leu Phe Val Gly Ser Ile Val Ile Gly Gly
    130                 135                 140

Tyr Ser Leu Phe Lys Val Gly Phe Gln Asn Leu Ile Arg Phe Asp Phe
145                 150                 155                 160

Asp Met Lys Thr Leu Met Thr Val Ala Val Ile Gly Ala Thr Ile Ile
                165                 170                 175

Gly Lys Trp Ala Glu Ala Ser Ile Val Val Ile Leu Phe Ala Ile Ser
            180                 185                 190

Glu Ala Leu Glu Arg Phe Ser Met Asp Arg Ser Arg Gln Ser Ile Arg
        195                 200                 205
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Ser Leu Met Asp Ile Ala Pro Lys Glu Ala Leu Val Arg Arg Asn Gly
    210                 215                 220

Gln Glu Ile Ile Ile His Val Asp Asp Ile Ala Val Gly Asp Ile Met
225                 230                 235                 240

Ile Val Lys Pro Gly Glu Lys Ile Ala Met Asp Gly Ile Ile Val Asn
                245                 250                 255

Gly Leu Ser Ala Val Asn Gln Ala Ala Ile Thr Gly Glu Ser Val Pro
            260                 265                 270

Val Ser Lys Ala Val Asp Asp Glu Val Phe Ala Gly Thr Leu Asn Glu
        275                 280                 285

Glu Gly Leu Ile Glu Val Lys Ile Thr Lys Tyr Val Glu Asp Thr Thr
    290                 295                 300

Ile Thr Lys Ile Ile His Leu Val Glu Glu Ala Gln Gly Glu Arg Ala
305                 310                 315                 320

Pro Ala Gln Ala Phe Val Asp Lys Phe Ala Lys Tyr Tyr Thr Pro Ile
                325                 330                 335

Ile Met Val Ile Ala Ala Leu Val Ala Val Val Pro Pro Leu Phe Phe
            340                 345                 350

Gly Gly Ser Trp Asp Thr Trp Val Tyr Gln Gly Leu Ala Val Leu Val
        355                 360                 365

Val Gly Cys Pro Cys Ala Leu Val Ile Ser Thr Pro Ile Ser Ile Val
    370                 375                 380

Ser Ala Ile Gly Asn Ala Ala Lys Lys Gly Val Leu Val Lys Gly Gly
385                 390                 395                 400

Val Tyr Leu Glu Lys Leu Gly Ala Ile Lys Thr Val Ala Phe Asp Lys
                405                 410                 415

Thr Gly Thr Leu Thr Lys Gly Val Pro Val Val Thr Asp Phe Glu Val
            420                 425                 430

Leu Asn Asp Gln Val Glu Glu Lys Glu Leu Phe Ser Ile Ile Thr Ala
        435                 440                 445

Leu Glu Tyr Arg Ser Gln His Pro Leu Ala Ser Ala Ile Met Lys Lys
    450                 455                 460
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Ala Glu Gln Asp Asn Ile Pro Tyr Ser Asn Val Gln Val Glu Glu Phe
465                 470                 475                 480

Thr Ser Ile Thr Gly Arg Gly Ile Lys Gly Ile Val Asn Gly Thr Thr
                485                 490                 495

Tyr Tyr Ile Gly Ser Pro Lys Leu Phe Lys Glu Leu Asn Val Ser Asp
            500                 505                 510

Phe Ser Leu Gly Phe Glu Asn Asn Val Lys Ile Leu Gln Asn Gln Gly
        515                 520                 525

Lys Thr Ala Met Ile Ile Gly Thr Glu Lys Thr Ile Leu Gly Val Ile
    530                 535                 540

Ala Val Ala Asp Glu Val Arg Glu Thr Ser Lys Asn Val Ile Gln Lys
545                 550                 555                 560

Leu His Gln Leu Gly Ile Lys Gln Thr Ile Met Leu Thr Gly Asp Asn
                565                 570                 575

Gln Gly Thr Ala Asn Ala Ile Gly Thr His Val Gly Val Ser Asp Ile
            580                 585                 590

Gln Ser Glu Leu Met Pro Gln Asp Lys Leu Asp Tyr Ile Lys Lys Met
        595                 600                 605

Gln Ser Glu Tyr Asp Asn Val Ala Met Ile Gly Asp Gly Val Asn Asp
    610                 615                 620

Ala Pro Ala Leu Ala Ala Ser Thr Val Gly Ile Ala Met Gly Gly Ala
625                 630                 635                 640

Gly Thr Asp Thr Ala Ile Glu Thr Ala Asp Ile Ala Leu Met Gly Asp
                645                 650                 655

Asp Leu Ser Lys Leu Pro Phe Ala Val Arg Leu Ser Arg Lys Thr Leu
            660                 665                 670

Asn Ile Ile Lys Ala Asn Ile Thr Phe Ala Ile Gly Ile Lys Ile Ile
        675                 680                 685

Ala Leu Leu Leu Val Ile Pro Gly Trp Leu Thr Leu Trp Ile Ala Ile
    690                 695                 700

Leu Ser Asp Met Gly Ala Thr Ile Leu Val Ala Leu Asn Ser Leu Arg
705                 710                 715                 720

Leu Met Arg Val Lys Asp Lys
                725
```

Claims

1. Genetically modified plants and plant cells, comprising nucleotide sequences encoding one or more heterologous heavy metal transporters or sequestration proteins.

2. Genetically modified plants or plant cells, the nucleotide sequence encoding the heterologous heavy metal transport proteins being genes encoding heavy metal transporters, such as transporters selected from the group consisting of P-type ATPase, 3 components efflux pumps or ABC transporters.

3. Genetically modified plants or plant cells according to the claim 2, **characterised in that** the nucleotide sequence encodes for cadmium ATPase.

4. Genetically modified plants or plant cells according to the claim 2 or 3, wherein the nucleotide sequence is *cad A* or a portion thereof allowing heavy metal transport.

5. Genetically modified plants or plant cells according to the claim 1, **characterised in that** the nucleotide sequence encoding the heavy metal sequestration protein belongs to the copA family.

6. Use of the genetically modified plants or plant cells according to any of the preceding claims for phytoremediation of contaminated sites, especially for the revegetation, phytostabilisation, phytoextraction of soils and/or water contaminated with trace elements.

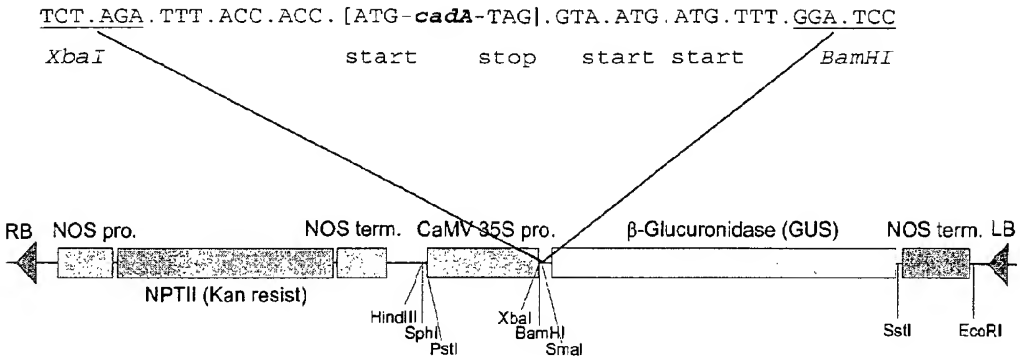

Fig. 1

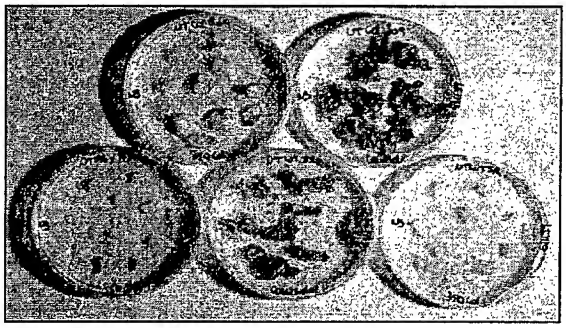

Fig. 2

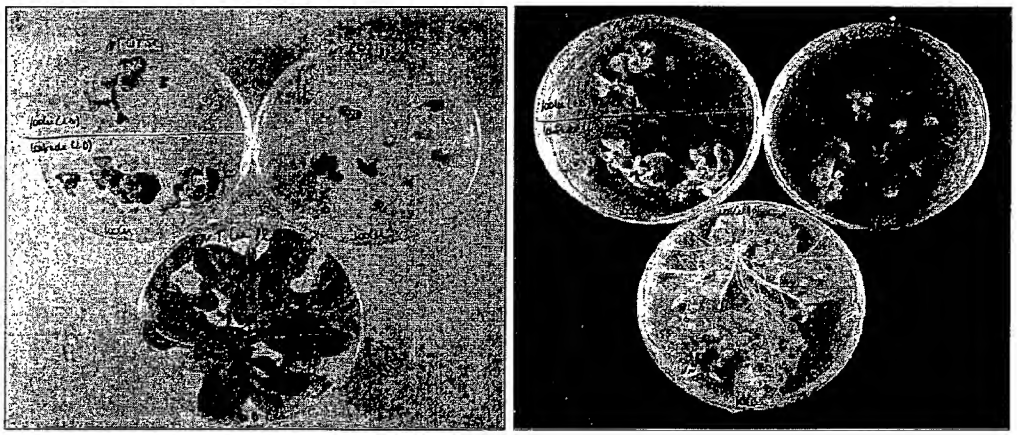

Fig. 3

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 00 87 0051

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	OW, D.W.: "Heavy metal tolerance genes: prospective tools for bioremediation" RESOURCES, CONSERVATION AND RECYCLING, vol. 18, no. 1, 1 November 1996 (1996-11-01), pages 135-149, XP004016621	1,2,6	C12N15/31 C12N15/82 C07K14/195 A01H5/00
A	* abstract * * page 137, line 1 - line 12 * * page 138, line 32 - page 140, line 7 * * page 147, line 16 - page 148, line 15 * ---	3,4	
X	WO 00 04760 A (THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA) 3 February 2000 (2000-02-03)	1,2	
A	* abstract * * page 7, line 1 - line 5 * * page 8, line 18 - page 9, line 10 * * page 9, line 25 - line 32 * * page 11, line 1 - line 7 * * page 15, line 3 - line 12 * * page 41 - page 47; example 4 * * page 55 - page 58; claims 1,2,9,10,15,16,26 * ---	3,4,6	
X	WO 97 45000 A (TRUSTEES OF DARTMOUTH COLLEGE; REGENTS OF THE UNIVERSITY OF MINNESOTA) 4 December 1997 (1997-12-04)	1,6	
A	* abstract * * page 2, line 4 - page 7, line 14 * * page 10, line 1 - page 13, line 14 * * page 36, line 34 - page 38, line 23 * * page 108 - page 110; claims 25,26,30,31,40,41,46-49 * --- -/--	2-4	

TECHNICAL FIELDS SEARCHED (Int.Cl.7)
C12N
C07K
A01H

~~The present search report has been drawn up for all claims~~

Place of search	Date of completion of the search	Examiner
BERLIN	28 August 2000	Fuchs, U

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

European Patent Office

Application Number
EP 00 87 0051

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:

☒ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

1 and 6 partially and 2-4 completely

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 00 87 0051

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	WO 98 04700 A (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 5 February 1998 (1998-02-05)	1,6	
A	* abstract * * page 1, line 8 - line 20 * * page 2, line 3 - line 7 * * page 2, line 30 - page 3, line 19 * * page 7, line 22 - line 24 * * page 8, line 28 - page 9, line 9 * * page 16, line 26 - page 18, line 12 * * page 26 - page 28; claims 1,5-7,10-12,14-19,23-26 *	2-4	
D,A	NUCIFORA, G. ET AL.: "Cadmium resisance from Staphylococcus aureus plasmid pI258 cad A gene results from a cadmium-efflux ATPase" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 86, no. 10, May 1989 (1989-05), pages 3544-3548, XP000938702 * the whole document *	1-4,6	TECHNICAL FIELDS SEARCHED (Int.Cl.7)
A	SILVER, S. & PHUNG, L.T.: "Bacterial Heavy Metal Resistence: New Surprises" ANNUAL REVIEW OF MICROBIOLOGY, vol. 50, 1996, pages 753-789, XP000925885 * the whole document *	1-4,6	

~~The present search report has been drawn up for all claims~~

Place of search	Date of completion of the search	Examiner
BERLIN	28 August 2000	Fuchs, U

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

European Patent Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 00 87 0051

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. Claims: 1 and 6 partially and 2-4 completely

   Genetically modified plants and plant cells, comprising nucleotide sequences encoding one or more heterologous heavy metal transporters; said nucleotide sequences encoding heavy metal transporters selected from the group consisting of P-type ATPases, 3 components efflux pumps or ABC transporters; said nucleotide sequence encoding cadmium ATPase; said nucleotide sequence being cadA or a portion therof allowing heavy metal transport and use of said plants or plant cells for phytoremediation of contaminated sites, revegetation, phytostabilisation, phytoextraction of soils / water contaminated with trace elements.

2. Claim : 1 and 6 partially and 5 completely

   Genetically modified plants and plant cells, comprising nucleotide sequences encoding one or more heterologous heavy metal sequestration proteins; said nucleotide sequences encoding heavy metal sequestration proteins belonging to the copA family and use of said plants or plant cells for phytoremediation of contaminated sites, revegetation, phytostabilisation, phytoextraction of soils / water contaminated with trace elements.

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 00 87 0051

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

28-08-2000

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0004760 A	03-02-2000	NONE	
WO 9745000 A	04-12-1997	AU 1142397 A	05-01-1998
		CA 2187728 A	29-11-1997
		US 5846821 A	08-12-1998
WO 9804700 A	05-02-1998	AU 3741597 A	20-02-1998
		US 5965792 A	12-10-1999

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82